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# Studies of the Prevention of Silica Urolithiasis

Dong-Hao Lu

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BY

**DONG-HAO LU**

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**Studies of the Prevention of Silica Urolithiasis**

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Dr. Royce J. Emerick

Thesis Advisor Date

Dr. David C. Hilderbrand

Head, Department of Chemistry Date

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# Studies of the Prevention of Silica Urolithiasis

## INTRODUCTION

Urethral obstruction by silica calculi is a major cause of death of cattle and sheep grazing western ranges of the United States. Similar calculi problems have been found in several other countries including Canada, U.S.S.R. and Australia. Range grasses having silica contents of 4 to 7% (dry basis) appear to be the silica source. In addition, silica urinary calculi have been reported to occur in dogs (30) from silicon of unknown origin, humans (39) consuming large quantities of silicate antacids and rats (32) and guinea pigs (78) experimentally fed tetraethylorthosilicate (TES).

Emerick and coworkers (33, 80) have found that supplemental dietary phosphate and acid-forming salts provide rats some protection from silica urolithiasis. This protective effect was beyond those effects attributable to increases in water intake and urine volume.

The objectives of my thesis research were (1) to study the calculi protective effect of dietary phosphorus using an animal model consisting of laboratory rats fed

diets containing 2% tetraethylorthosilicate (TES) [ $(C_2H_5O)_4Si$ ], (2) to study *in vitro* the effect of phosphorus on precipitation of a polysilicic acid-protein complex which is believed to be important to the formation of silica calculi, and (3) to develop a ruminant model for future silica urinary calculi studies.

# REVIEW OF LITERATURE

## CHEMICAL AND PHYSICAL CHARACTERISTICS OF SILICON

The chemistry of silicon. Silicon is the second most common element on the surface of the earth, but it occurs only in combination with other elements. Recently it has been recognized that soluble silica, previously known to be essential for development of certain invertebrates, plays a role in vertebrate bone formation (27, 81).

While in solution in water, silica is largely in a monomeric form (containing only one silicon atom). Normally the formula is $Si(OH)_4$. The hydrated state of silica is not known, but some scientists believe that each OH group links to one water molecule by hydrogen bonding, and the hydrated molecule may be represented as $Si(OH:OH_2)_4$ (92).

The structure of monosilicic acid involves silicon coordinated with four oxygen atoms, as in amorphous vitreous silica and in crystalline quartz. It is essentially nonionic in neutral and weakly acidic solutions, and it is not transported by electric current unless ionized in an alkaline solution. It can not be salted out of water; it also can not be extracted by neutral organic solvents (43).

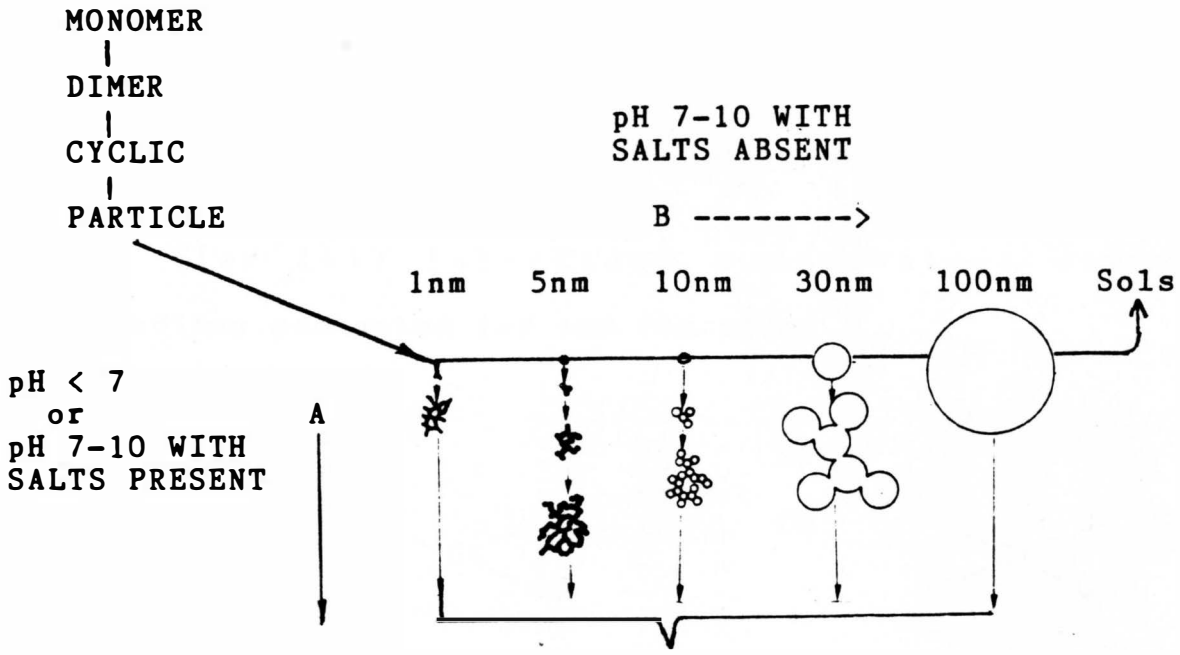
Silica remains in the monomeric state for long periods in water at 25°C at concentrations less than about 100 ppm (as $SiO_2$). At higher concentrations, it initially polymerizes to form polysilicic acids of low molecular weight and then larger polymeric species recognized as colloidal particles. The rate of the polymerization is proportional to the square of the concentration during the time that monomeric silicic acid exceeds saturation (41).

Figure 1-1 shows the succeeding steps in polymerization of silica from monomer to large particles and gels as presented schematically by Iler (43).

At 25°C, if the concentration of monosilicic acid is less than 100 ppm (as $SiO_2$), the acid is soluble and stable. But if the concentration becomes greater than 100 to 200 ppm and there is no solid phase existing on which the soluble silica might be deposited, the monomer polymerizes by condensation reactions to form the dimer and polymer of silicic acid.

$OH^-$ ion or $H^+$ ion is involved in the reaction mechanism. The reaction rate is proportional to the concentration of $OH^-$ ion above pH 2, and to the $H^+$ ion below pH 2. At around pH 2 , the rate of polymerization is at a minimum. It has been suggested that since pH 2 is the isoelectric point of silica, the catalyst below pH 2 is the $H^+$ ion which forms an active cationic complex, and

Figure 1-1. Polymerization behavior of silica[1]




[1]In basic solution (B), particles in sol grow in size with decrease in numbers; in acid solution or in presence of flocculating salts (A), particles aggregate into three-dimensional networks and form gels (43).

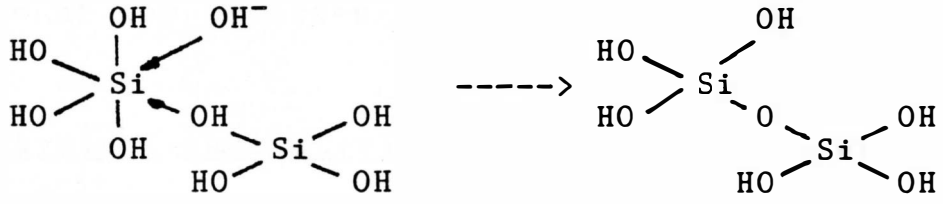
* * *

above pH 2 it is the $OH^-$ ion which helps to form an active anionic silica (43).

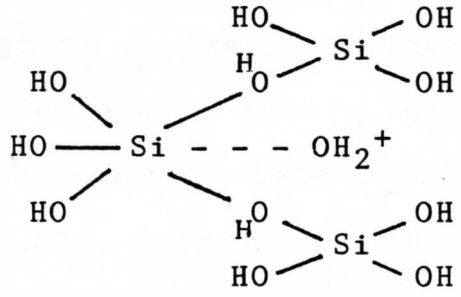
The self-condensation of the silicic acid monomer catalyzed by $OH^-$ ion is believed to be as follows:

$$2\ Si(OH)_4 \xrightarrow{OH^-} (HO)_3SiOSi(OH)_3 + H_2O$$

Iler (41) introduced a 6-covalent stage intermediate mechanism for the reaction:



For this reaction (above pH 2), the rate of disappearance of monomer is a second order reaction. However, when pH is below 2, Okkerse (70) believed that the rate of disappearance of monomer is a third order reaction, involving a three-silicon intermediate as follows:



Polymerized silica has a tendency to have a maximum of siloxane (Si-O-Si) bonds and a minimum of uncondensed SiOH groups. Therefore, at the earliest stage of polymerization, condensation quickly leads to ring structures, followed by addition of monomer to these structures. Since small particles are more soluble than large ones, the larger size particles grow as the smaller ones dissolve and the silica is redeposited upon the larger ones. However, this is a slower process and may be negligible at low pH after the monomer has been depleted (89).

Physical characteristics of silicon. Silicon is one of the trace elements "essential" for higher animals. This conclusion was reported in 1972 by Carlisle (27) with the establishment of a deficiency state in chicks incompatible with normal growth, and the contrasting normal growth on a diet containing a silicon supplement. About the same time, Schwarz (81) developed an all-plastic, trace element-controlled isolator system for small laboratory animals and found silicon to be required for growth in the rat. Silicon was subsequently shown to have a role in bone matrix formation and bone growth (27).

The form of dietary silicon is an important factor affecting its absorption. Absorption relates to the rate of formation of soluble or absorbable silicon in the

gastrointestinal tract (28). Excess amounts of certain other mineral elements in the diet may also affect silicon absorption. This is possibly related to a reduction in the formation of soluble silicon (28).

Normally the whole-blood levels of silicon in humans, monkeys and rats average approximately 1 ug/ml, but ovines have higher values approximating 5 ug/ml (28). The highest silicon concentrations normally are in connective tissues such as aorta, trachea, tendon, bone, skin and its appendages. In the body, silicon is freely diffusible throughout tissue fluid (28). Policard et al. (73) suggested that polymeric molecules of silicic acid containing up to four or five silicon-oxygen units characterize the transport form of silicic acid in blood.

Jones and Handreck (47) found that the amounts of silica excreted in sheep urine increased when silica content of the diet was increased in the range of 0.10 to 2.84 %, but reached a maximum of 205 mg $SiO_2$ per day, this amount representing < 4% of the total intake. Similar results have been shown by others in humans (28), rats (52), guinea pigs (78), sheep and cattle (2).

The maximum urinary silica concentration that may be reached is dependent on the rate and extent of silicon absorption from the gastrointestinal tract into the blood. In the ruminant, the solubility of silica in the rumen

fluid also affects the values. Silicon must pass rapidly into the urine and tissues after it has entered the bloodstream because the silicon level in the blood remains relatively constant even when intakes of silicon are much different (28).

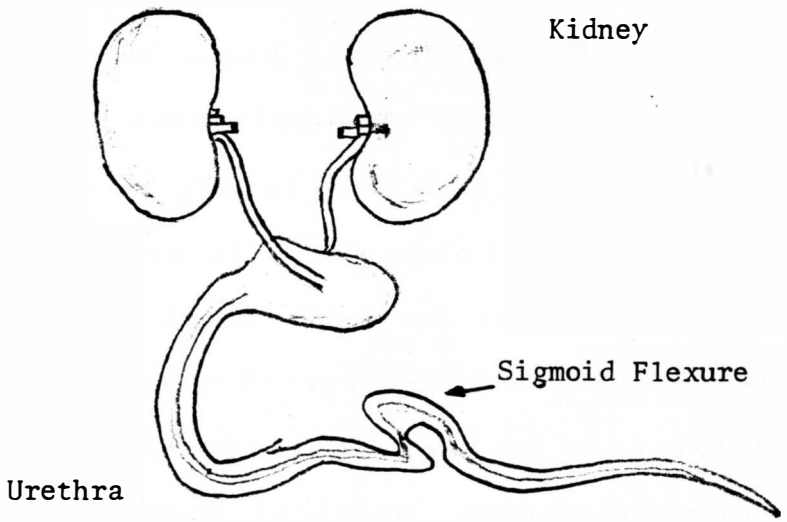
Excess silicon has its negative effects on animals. One of the problems is silica urolithiasis which is the subject of this research and will be discussed later. Another problem is silicosis in humans. Silicosis occurs in certain classes of miners as a result of the continued inhalation of silica particles into the lungs. This subject has been recently reviewed by Carlisle (28). Particles of silica and asbestos (fibrous silicates of complex composition) can stimulate a severe fibrogenic reaction in the lungs and elsewhere in the body. This reaction arises initially from phagocytosis of silica particles by alveolar macrophages. The basis for toxicity of silica to macrophages is that the particles are taken up into lysosomes and readily damage lysosomal membranes through hydrogen-bonding reactions. Silicon in the form of asbestos may cause the formation of malignant tumors of the pleura and peritoneum, but it is believed that physical shape and size of the particles is an important factor (28).

## SILICON URINARY CALCULI

General features of silica urinary calculi. Urinary silica is generally readily excreted, but in some instances part of it is deposited in the kidneys, bladder, or urethra to form calculi. Small calculi may be excreted without any problem, but large calculi may block the passage of urine and cause death. In many areas the presence of silica urinary calculi is a common condition. Connell et al. (29), Whiting et al. (91) and Bailey (18) reported that 50% to 90% of range animals in some areas developed latent silica calculi. Blockage, when it occurs, is usually at or near the urethral sigmoid flexure (Figure 1-2). Upon rupture of the bladder or urethra, urine enters the abdominal cavity or infiltrates the tissues of the ventral abdomen. Subsequent death will occur unless timely surgery is performed.

In ruminants, silica in urinary calculi is from the diet. In the northern Great Plains of North America and some areas of Australia, indigenous grasses contain silica approximating 4% to 7% of the dry matter (18, 33). The level of silica in the grasses varies with the change of seasons. Usually, in spring the grasses contain relatively low levels of silica and high levels of protein. Silica increases and protein decreases as the

Figure 1-2. The urinary tract of the male sheep[1]




[1]Arrows indicate the points where urinary calculi most often cause blockage.

season advances with highest silica concentrations occurring in winter (2). Range animals ingest large quantities of silica from native range hay. For example, yearling steers may consume up to 10 kg/head daily of grass dry matter which contains about 500 g of silica (12, 13). Sheep in Australia have been estimated to consume as much as 20 to 40 g/head daily of silica (48, 66).

Bailey et al. (10) examined 74 calculi which had caused urethral obstruction in range cattle. The calculi ranged in diameter from 2 to 10 mm and in weight from 20 to 558 mg. They were described as hard and stoney, from white to dark brown in color, round, ovoid or mulberry in shape. The average composition of these calculi were 50.7% silica, 4.7% calcium, 13.8% calcium oxalate and 26.1% organic matter. In general, the silica calculi from range cattle and sheep have usually contained about 75% silica, 20% organic matter, 2.5% nitrogen, 2% water and small amounts of other mineral components (7, 20, 51, 63, 85). Keeler et al. (50, 53) found that the organic matrix of siliceous calculi from cattle contained 16 amino acids plus galactose, mannose, fucose, rhamnose and glucose, but not hexuronic acid and only traces of sialic acid.

Most silica calculi can be sorted into three categories composed of three distinct types of material: (1) Friable, amorphous silica, poor in organic matter;

this type can be found at the core of many calculi and occasionally interspersed between overlying laminations. (2) Dense, concentric laminations, high in silica; this type can be found in the bulk and the outer regions of some calculi. (3) Poorly organized material, high in organic matter and usually located between the core and outer laminations (7, 19, 51).

In cattle, silica calculi can be found in both sexes, but obstruction usually occurs only in males. Females have a shorter, wider urethra than males which allows the passage of calculi before they reach obstructive size (29, 62, 91). Obstruction is more common in steers than in bulls because castration retards urethral development (11, 59).

Mechanism of Silica Calculus Formation. The mechanism of silica calculus formation in animals is not known, but some researchers believe that it may involve the precipitation of polymerized silicic acid by urine protein (8, 18, 51).

Silicic acid in pure solution at concentrations normally found in urine (200 to 700 ppm) and at physiological pH does not form a precipitate easily. However, in some studies in vitro, proteins, including bovine urine protein, added to polymerized silicic acid produced insoluble polysilicic acid-protein complexes (8,

21, 40). The rate of precipitation was higher at pH 5.5 than at 6.5 and 7.5 and varied depending on the nature and concentration of the electrolytes present (8). Allison (1) suggested that the mechanism was hydrogen bonding between surface silanol groups and the secondary amides of the proteins.

Bailey (18) suggested that the deposition of silica in the process of calculus formation is due to a mechanism similar to that observed for the deposition of silica *in vitro*. The first step is described as formation of masses of aggregated silica micelles. When the starting concentration of silicic acid is high, a sol is formed from polymerized silicic acid, and spherical micelles of approximately 150 to 300 nm diameter are formed (45). The presence of electrolytes cause flocculation of micelles into porous aggregates which are similar to the amorphous silica found in siliceous calculi. In the second step, aggregated silica micelles act as nuclei, and silica is continually deposited on it (18).

## PREVENTION OF SILICA URINARY CALCULI

Several methods have been used to prevent the formation of silica urinary calculi. The measures of prevention can be sorted into the following three types.

Reducing the problem feeds. Reducing intake of feeds such as native range hay containing a high level of silica, and giving relatively low silica supplements such as alfalfa hay or corn grain may by beneficial. However, this usually is not practical because of such things as range location and nonavailability of other sources of feed.

Increasing urinary volume. There is a hypothesis that a low output of water in urine relative to the output of silicic acid is a necessary condition for silica calculi formation (18). The urinary concentration of silicic acid can be reduced by increasing water intake.

Methods that have been used by Bailey (18) for increasing water intake include incorporating 4% sodium chloride in the diet; supplying ad libitum feed supplements containing 15 to 25% salt (NaCl); giving extra water by stomach tube. On average, water intakes under these conditions were 40% greater than the control group, and the concentration of silicic acid in urine was generally reduced below saturation. The results of these experiments showed that the formation of urinary calculi was suppressed by the higher water intake. About 1 g salt/kg body weight was required per day to reduce calculus formation to negligible levels (3, 5, 9).

Supplying dietary supplements of chloride,

phosphate or urine acidifying salts. Using an animal model consisting of rats fed diets containing the calculogenic agent, tetraethylorthosilicate (TES), Emerick (33) showed that increases in dietary sodium chloride provided some protection from silica urinary calculi. This benefit from sodium chloride was obtained without increases in urine volume. Sodium sulfate at levels equivalent to the sodium chloride treatment proved to be ineffective in reducing the incidence of silica urinary calculi.

Emerick (33) further demonstrated a protective effect for supplemental dietary phosphates. Later, Schreier and Emerick (80) showed that the protection afforded by supplemental phosphorus was diminished or negated by supplemental calcium or a urine alkalizing salt ($NaHCO_3$), but protection was increased by the urine-acidifying salt, ammonium chloride. These researchers concluded that the beneficial effects of dietary phosphorus and urine-acidifying salts were not dependent upon increases in urine volume. However, because some of the phosphate compounds tended to have a urine acidifying effect, a portion of the protection attributed to supplemental phosphorus may have been due to urine acidification. The basal diet used by these researchers yielded urine pH approximating 6.5. Substituting egg

albumin for the dietary casein yielded urine pH of about 7.5. Reductions in silica urolithiasis from the feeding of supplemental phosphorus were achieved with both diets, indicating that the anticalculogenic effect of phosphorus was not entirely dependent upon an acidic urine.

# MATERIALS AND METHODS

## SYNERGISM OF DIETARY PHOSPHATE AND URINE ACIDIFYING SALTS IN PREVENTING SILICA UROLITHIASIS IN RATS

In the experiment, a subprophylactic concentration of ammonium chloride was factored with three levels of supplemental phosphorus to determine whether the antiurolithic effects of dietary phosphate and urinary acidifying salt are synergistic.

The model system described by Emerick et al. (32) using Sprague-Dawley male rats fed diets containing 2% of (TES) was used. One hundred and twenty male Sprague-Dawley rats (Sasco, Inc., Omaha, NE) having an initial average weight of 54.0 $\pm$ 0.9 g were randomly allotted across the six treatments in the experiment. There were 20 rats per treatment. Rats were housed individually in hanging stainless steel cages with wire mesh floors in a room maintained at 23-25°C and lighted 12 h/d (0600-1800). Deionized water and diet were fed ad libitum.

The basal diet was the same as in the model system described by Emerick (33) except that spray-dried egg albumin was substituted for casein to obtain a lower concentration of dietary phosphorus and to increase urine alkalinity. The spray-dried egg albumin was autoclaved at 121°C for 1 h and dried at 120°C for 1 h prior to use.

The experimental diets are shown in Table 2-1. Additions to the basal diet, representing the dietary treatments, were made by substituting for dextrose. All diets contained 2% of TES (Baker Chemical Co., Phillipsburg, NJ). Six dietary treatments were used in a 2 x 3 factorial arrangement with two concentrations of ammonium chloride, 0 and 0.067 equivalents/kg of diet, and three concentrations of dietary phosphorus, 0, 0.15% and 0.30% from $Na_2HPO_4$. The three concentrations of supplemental phosphorus yielded total dietary phosphorus concentrations of 0.29%, 0.44% and 0.59%, respectively.

The analysis of variance utilized a model consisting of ammonium chloride and phosphorus main effects, and ammonium chloride x phosphorus interaction.

The rats were weighed weekly during the 8-wk experiment. Water intake was measured at 2, 4 and 6 wk for a 48-h period. Water intake/24 h was expressed as one-half of the 48-h volume.

Stainless steel metabolism cages were employed to obtain 24-h urine collections at 3, 5 and 7 wk. Urine was collected in plastic tubes containing 1 ml of toluene. Urine pH was determined with a combination glass electrode on the fresh urines. Blood samples were obtained at termination of each experiment by cardiac puncture from rats anesthetized with Halothane (Abbot Laboratories,

Table 2-1

Diet Composition For Rat Experiment

| Ingredient | (1) % | (2) % | (3) % | (4) % | (5) % | (6) % |
|---|---|---|---|---|---|---|
| Dextrose, anhydrous | 67.0 | 66.3 | 65.5 | 66.6 | 66.0 | 65.3 |
| Egg Albumin | 20 | 20 | 20 | 20 | 20 | 20 |
| Salt Mixture[1] | 4 | 4 | 4 | 4 | 4 | 4 |
| Vitamin Mix[2] | 2 | 2 | 2 | 2 | 2 | 2 |
| Corn Oil | 5 | 5 | 5 | 5 | 5 | 5 |
| TES | 2 | 2 | 2 | 2 | 2 | 2 |
| $NH_4Cl$ | 0 | 0 | 0 | 0.36 | 0.36 | 0.36 |
| $Na_2HPO_4$[3] | 0 | 0.69 | 1.37 | 0 | 0.69 | 1.37 |

[1]The percent composition of salt mixture (Salt Mixture P-H, ICN Nutritional Biochemicals, Cleveland, OH): dipotassium phosphate, 32.2; calcium carbonate, 30.0; sodium chloride, 16.7; magnesium sulfate (hydrate), 10.2; manganese sulfate, 0.51; calcium phosphate monohydrate, 7.5; ferric citrate, 2.75; potassium iodide, 0.08; copper sulfate, 0.03; zinc chloride, 0.025; cobalt chloride, 0.005. An

(notes of Table 2-1, continued)

additional 0.083 % zinc chloride was added to the commercially prepared salt mixture.

[2]Vitamin mixture composition in gram/kg (Vitamin diet fortification mixture, ICN Nutritional Biochemicals, Cleveland, OH) vitamin A, 4.5; vitamin D, 0.25; alpha tocopherol, 5.0; ascorbic acid, 45; inositol, 5.0; choline chloride, 75; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine, hydrochloride, 1.0; thiamine hydrochloride, 1.0; calcium pantothenate, 3.0; biotin, 0.20; folic acid, 0.90; vitamin $B_{12}$, 0.00135.

[3]0.687% $Na_2HPO_4$ is equivalent to 0.15% phosphorus added to diet; 1.374 % $Na_2HPO_4$ is equivalent to 0.30% phosphorus added to diet.

* * *

Chicago, IL). Blood was placed in heparinized tubes. Plasma was removed by centrifugation and stored at -25°C until analyzed. The rats were killed by excess anesthetization, and the urinary bladder and kidneys of each rat were examined for the presence of uroliths.

Uroliths were cleaned, air-dried, weighed, and pooled by treatment for analysis. Ten-mg powdered calculi samples were placed in platinum crucibles previously cleaned with molten sodium carbonate, and they were ashed over a bunsen burner. The ashed samples were fused with 0.5 g of sodium carbonate. After cooling, the melt was dissolved in 10 ml of water and a minimum of HCl. Aliquots were removed and analyzed for silica by the method described below for urine silica.

Calcium and magnesium concentrations in urine and plasma were determined by atomic absorption spectrophotometry (Perkin-Elmer model 503, Norwalk, CT) in the presence of 0.5% (w/v) lanthanum. Urine silica was measured by a silicomolybdate method as modified by Emerick (32). Urine and plasma phosphorus were determined using the Fiske and Subbarow phosphomolybdate method (71).

## IN VITRO STUDIES ON THE EFFECT OF PHOSPHORUS ON PRECIPITATION OF A POLYSILICIC ACID-PROTEIN COMPLEX

The effect of phosphorus on precipitation of a

polysilicic acid-protein complex was studied with four concentrations of phosphorus and three pH levels at a constant ionic strength.

In the experiment, the protein source was bovine serum albumin (crystallized and lyophilized, Sigma Chemical Co., St. Louis, MO). The stock solutions of pH 6.0, 6.5 and 7.0 phosphate buffers were produced so that when they were diluted 1:20 with the silica solution, a constant ionic strength of 0.1724 N was obtained. Concentrations of the monobasic sodium phosphate monohydrate and dibasic sodium phosphate salts in the stock buffer solutions are shown in Table 2-2.

By acid hydrolysis of TES (J. T. Baker Chemical Co., Phillipsburg, NJ 08865) at pH 3 (0.001 N HCl), a 600 ppm $SiO_2$ solution was prepared for use as a source of silica in this study. The level was established based on previous rat studies that showed the urine of TES-fed rats to contain about 600 ppm $SiO_2$. The $SiO_2$ solution was neutralized to a pH of 7.0 with 0.1 N NaOH, and silica was allowed to polymerize overnight.

Four concentrations of phosphate including 0.1724, 0.0862, 0.0431 and 0.0216 N were studied at pH 6.0, 6.5 and 7.0. The most concentrated phosphate solution was obtained by diluting 3 ml of the appropriate stock phosphate solution for the desired pH with 57 ml of the

aged $SiO_2$ solution. The more dilute phosphate solutions were obtained by replacing a portion of the 3 ml phosphate solution with an equal volume of the 3.45 N NaCl solution. This procedure provided a constant ionic strength of 0.1724 equivalents/L in all reaction mixtures. The reaction mixtures used in this experiment are shown in Table 2-3.

All reactions were conducted in glass flasks. When necessary, solutions were adjusted to the correct pH with dilute NaOH or HCl at the start of each trial. Duplicate 10 ml aliquots of each of the reaction mixtures were mixed with 1.0 ml of 1% bovine serum albumin. The mixture was shaken, and after 15 min turbidity was measured in a spectrophotometer (Spectronic 88, Bausch and Lomb, Rochester, NY) at a wavelength of 440 nm.

## DEVELOPMENT OF A SHEEP MODEL FOR THE STUDY OF SILICA URINARY CALCULI

This experiment represents an attempt to develop a ruminant model for future studies of silica urolithiasis.

Sixty six lambs, initial average weight of 39.1 $\pm$ 4.5 kg, were used as experimental animals. The experiment was conducted during summer, from July to September, 1986. The lambs were randomly allotted to six outdoor pens representing three treatments. There were 22 lambs per

Table 2-2

Composition of the Stock Buffer Solutions

| pH | $Na_2HPO_4$ (g/100ml) | $NaH_2PO_4.H_2O$ (g/100ml) |
|---|---|---|
| 6.0 | 5.29 | 32.15 |
| 6.5 | 9.80 | 18.90 |
| 7.0 | 13.50 | 8.20 |

Table 2-3

Composition of the Reaction Mixtures

| Sodium phosphate ionic strength (N) | 600 ppm silicic acid (ml) | Stock NaCl solution[1] (ml) | Stock phosphate solution[2] (ml) |
|---|---|---|---|
| 0.1724 | 57 | 0.0 | 3.0 |
| 0.0862 | 57 | 1.5 | 1.5 |
| 0.0431 | 57 | 2.25 | 0.75 |
| 0.0216 | 57 | 2.625 | 0.375 |

[1]NaCl stock solution was 3.45 N.

[2]One of three stock solutions was used depending on the desired pH.

* * *

treatment and 11 lambs per pen. Six pens were in a single row from south to north. Each pen had 24 $m^2$ in area and 4.9 $m^2$ for feed bunk space.

The diet was the same for all pens and the composition is shown in Table 2-4.

The diet was fed ad libitum with an average daily consumption of 1.24 kg per sheep. The treatments consisted of acidic drinking waters containing 3 levels of silicic acid (0 ppm, 1500 ppm and 3000 ppm calculated as $SiO_2$). The waters were prepared daily. For the control group (0 ppm $SiO_2$ treatment), 681 ml 1N $H_2SO_4$ was added to 150 L of Brookings city water to attain a pH of 3.0. For the 3000 ppm $SiO_2$ treatment, 681 ml 1N $H_2SO_4$ and 1.67 L tetraethylorthosilicate (TES) (Baker Chemical Co., Phillipsburg, NJ) were added to 150 L Brookings city water and stirred with an electric stirrer for approximately 2 hours until hydrolysis was complete. This provided water having 3000 ppm of molybdate-reactive silica and a pH of 3.0. To obtain the 1500 ppm $SiO_2$ treatment water, equal amounts of the 0 and 3000 ppm $SiO_2$ drinking waters were mixed together. The drinking waters were given ad libitum. Water intake was measured for each pen on a weekly basis throughout the experimental period, and the average daily intake was calculated.

Metabolism cages were employed to obtain 24-h

Table 2-4

Composition of Diet for Sheep Experiment[1]

| Ingredient | Percent |
|---|---|
| Bromegrass hay | 60.0 |
| Corn | 37.1 |
| Urea | 1.0 |
| Limestone | 0.1 |
| Trace mineralized salt | 0.3 |
| Molasses | 1.5 |
| Vitamins A, D and E[2] | + |

[1]Bromegrass and the concentrate portions of the diet were fed together in the same bunk without deliberate mixing.

[2]Vitamin premix provided the following per kg of the concentrate portion of the diet: 2423 IU vitamin A, 485 IU vitamin D, 0.24 IU vitamin E.

urine collections during the third, fifth and eighth weeks of the experiment. Four lambs per pen at the third and fifth weeks and eight lambs per pen at the eighth week were randomly chosen for the urine collections. The urine samples were collected in plastic pails containing toluene and were filtered through four layers of cheese cloth.

At the end of the experimental period, blood samples were obtained from all lambs by jugular vein puncture. The blood was heparinized, and plasma was separated and stored frozen for later analysis. All lambs were killed at an area packing plant and the urinary bladders and kidneys were opened and examined for the presence of calculi. The calculi were cleaned, air-dried and weighed before further analysis.

Urine pH was determined with a combination glass electrode. Urine samples were analyzed for total silica by the silicomolybdate method described for the rat experiment. For the urine and blood plasma, phosphate was determined by the phosphomolybdate method of Fiske and Subbarow (71), and calcium and magnesium were determined by atomic absorption spectroscopy in the presence of 0.5% lanthanum.

Urinary calculi, pooled by treatment groups, were analyzed for percent ash and percent silica of the ash. Silica was determined on 10-mg samples of the pooled

calculi by the same method used in the rat experiment.

Data for incidence of urinary calculi were analyzed by chi-square. All other data were statistically analyzed by least squares means analysis of variance. Urine data for the three collection periods were pooled for statistical analysis.

During the experimental period, two lambs in one pen of the 1500 ppm $SiO_2$ treatment died from causes unrelated to the experiment.

# RESULTS AND DISCUSSION

## SYNERGISM OF DIETARY PHOSPHATE AND URINE ACIDIFYING SALTS IN PREVENTING SILICA UROLITHIASIS IN RATS

Data from this experiment are shown in Table 3-1. Emerick and Lu found 0.10 equivalents/kg diet of ammonium chloride was the minimum effective concentration for reduction of silica urolith formation in the rat model (35). Therefore, the concentration of 0.067 equivalents/kg diet of ammonium chloride, factored with three levels of dietary phosphorus supplementation in this experiment, was selected as being below the minimum effective concentration for reduction of silica urolithiasis. This low level of ammonium chloride, without the addition of supplemental phosphorus, again failed to reduce the incidence or weight of silica uroliths. None of the treatments had an effect on weight gains during the 8-week experimental period. Uroliths from all groups were found to contain 79% ash and to have silica contents approximating 100 % of the ash.

Urinary pH was decreased ($p < 0.01$) by dietary ammonium chloride. Mean urinary pH values of all ammonium chloride treatments were 7.14-7.16, and treatments without ammonium chloride had mean pH values of 7.44-7.53. Supplemental phosphorus had no significant effect on

Table 3-1. Effect of Supplemental $Na_2HPO_4$ factored with $NH_4Cl$ [1,2,3]

| | 0 $NH_4Cl$ | | | 0.067 equiv $NH_4Cl$ | | | |
|---|---|---|---|---|---|---|---|
| | 0% P | 0.15% P | 0.30% P | 0% P | 0.15% P | 0.30% P | SE |
| Wt gain, g | | | | | | | |
| 28 days | 153.2 | 151.2 | 158.4 | 158.4 | 150.3 | 153.7 | 4.4 |
| 56 days | 242.1 | 231.6 | 248.0 | 256.8 | 235.7 | 240.1 | 8.8 |
| Water intake [a] ml/24 hr | 34.4 | 36.5 | 40.4 | 36.6 | 35.2 | 37.0 | 1.2 |
| Urine data | | | | | | | |
| Volume, [b] ml/24 hr | 22.7 | 26.3 | 25.1 | 21.3 | 26.0 | 26.1 | 1.7 |
| pH [c] | 7.53 | 7.44 | 7.45 | 7.16 | 7.15 | 7.14 | 0.03 |
| $SiO_2$, [d] mg/dl | 60.2[i] | 51.3[j] | 60.4[i] | 60.7[k] | 58.4[km] | 50.8[m] | 3.0 |
| Calcium, [e,f] mg/dl | 12.9 | 8.8 | 5.5 | 16.2 | 6.7 | 6.8 | 1.1 |
| Magnesium, [e] mg/dl | 27.7 | 22.0 | 19.8 | 28.2 | 20.4 | 19.3 | 1.7 |
| Phosphorus, [g] mg/dl | 40.5 | 98.9 | 167.4 | 44.8 | 97.6 | 164.0 | 6.7 |
| Blood plasma | | | | | | | |
| Calcium, mg/dl | 10.1 | 9.8 | 10.1 | 10.3 | 10.3 | 10.3 | 0.09 |
| Magnesium, mg/dl | 1.8 | 1.7 | 1.8 | 1.8 | 1.8 | 1.8 | 0.05 |
| Phosphorus, mg/dl | 6.9 | 6.9 | 7.4 | 7.3 | 7.3 | 7.6 | 0.21 |
| Urolith incidence | 10/20[i] | 5/20[i] | 2/20[j] | 8/20[k] | 0/20[m] | 0/20[m] | |
| Urolith wt, [h] mg | 13.0 | 6.6 | 1.5 | 9.1 | -- | -- | 1.9 |

(Notes for Table 3-1, continued)

[1]Basal diet contained 0.29% phosphorus and 0.51% calcium; phosphorus levels (0, 0.15 and 0.30%) indicated in treatment headings are from additional $Na_2HPO_4$. [2]Initially 20 rats per treatment, average wt = 54.0 $\pm$ 0.9 g. [3]Statistical effects are by analysis of variance: [a]Phosphorus effect, $P<0.05$; 0.30% differs from 0% and 0.15%. [b]Phosphorus effect, $P<0.05$; 0.15% and 0.30% differs from 0.%. [c]$NH_4Cl$ effect, $P<0.01$. [d]Phosphorus x $NH_4Cl$ interaction, $P<0.01$. [e]Phosphorus effect, $P<0.01$; 0.15% and 0.30% differ from 0%. [f]Phosphorus x $NH_4Cl$ interaction, $P<0.05$. [g]Phosphorus effect, $P<0.01$; each level differs from all others. [h]Phosphorus effect, $P<0.05$; 0.3% differs from 0%. [i,j,k,m]Means within the same $NH_4Cl$ treatment not sharing a common superscript differ by the method of least significant differences except urolith incidences which differ by Chi-Square orthogonal analysis, $P<0.05$.

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urinary pH (Table 3-1). Thus, when compared with the 50% urolith incidence in the control group (no added phosphorus or ammonium chloride), reductions to 25% incidence by 0.15% added phosphorus ($P = 0.08$) and to 10% incidence by 0.30% added phosphorus ($P < 0.05$) can not be attributed to differences in urinary pH. However, an absence of uroliths in rats fed 0.15% or 0.30% supplemental phosphorus in combination with ammonium chloride indicates a greater antiurolith effectiveness for supplemental phosphorus when fed under conditions that also reduce urinary pH.

Ammonium chloride had no effect on water intake or urine volume. Rats fed 0.30% supplemental phosphorus had an average water intake that was 109% ($P < 0.05$) of the value for the control group. Average urine volume for the 0.15% phosphorus treatments was 119% and for 0.30% phosphorus it was 116% of the control value ($P < 0.05$). The increased urine volume associated with the feeding of supplemental phosphorus may have been partially responsible for its antiurolithic effectiveness in this instance. However, lower silica urolith incidences attributed to phosphorus supplementation in past rat experiments were not associated with larger urine volumes (33, 80).

Compared to the control group fed no supplemental

phosphorus, a lower urinary silica concentration occurred in the 0.15% phosphorus group in the absence of ammonium chloride ($P < 0.01$) and in the 0.30% phosphorus group in its presence ($P < 0.01$). No consistent relationship between urinary silica concentration and urolith incidence was apparent in the instance.

Supplemental phosphorus lowered ($P < 0.01$) urinary calcium and magnesium concentrations and increased ($P < 0.01$) urinary phosphorus. No ammonium chloride effect on urinary calcium concentration was observed, and supplemental phosphorus appeared to be about equally effective in reducing urinary calcium concentrations in the presence as in the absence of ammonium chloride.

Based on the data from this experiment, it is concluded that supplemental dietary phosphate and urinary acidifying salts independently reduce silica urolith formation and their effects appear to be synergistic.

Urinary effects observed in this experiment that may translate into reduced silica urolith formation include a lower pH due to the feeding of ammonium chloride, and lower calcium and magnesium and higher phosphorus concentrations from the feeding of supplemental phosphorus. The importance of silica concentration and ionic strength of the solution to silica polymerization (43), and therefore to urolith development, is a foregone

conclusion.

Richardson (77) has indicated that initial steps of silica polymerization, i.e., monomeric silica forming a polymeric sol, are more rapid at pH values approximating 7-9, but that gelation from the polymeric sol may require a pH less than 7. Proteins present in urine combine with polysilicic acids to form insoluble complexes (8, 51). Silica uroliths contain proteinous matrix materials, and precipitation of polysilicic acid-protein complexes has been indicated as a possible mechanism for their formation. We postulate that materials contributing to urine acidification tend to reduce the initial rate of polysilicic acid formation, and that urinary phosphorus has an inhibitory effect upon the formation of polysilicic acid-protein complexes, the extent of the inhibition depending somewhat on pH.

## IN VITRO STUDIES ON THE EFFECT OF PHOSPHORUS ON PRECIPITATION OF A POLYSILICIC ACID-PROTEIN COMPLEX

The amount of precipitate formed in this study was assumed to be proportional to the light absorbance measured by a spectrophotometer at 440 nm.

Table 3-2 and Figure 3-1 show the quantities of polysilicic acid-protein complex formed in solutions varying in phosphorus concentration and pH. Protein-

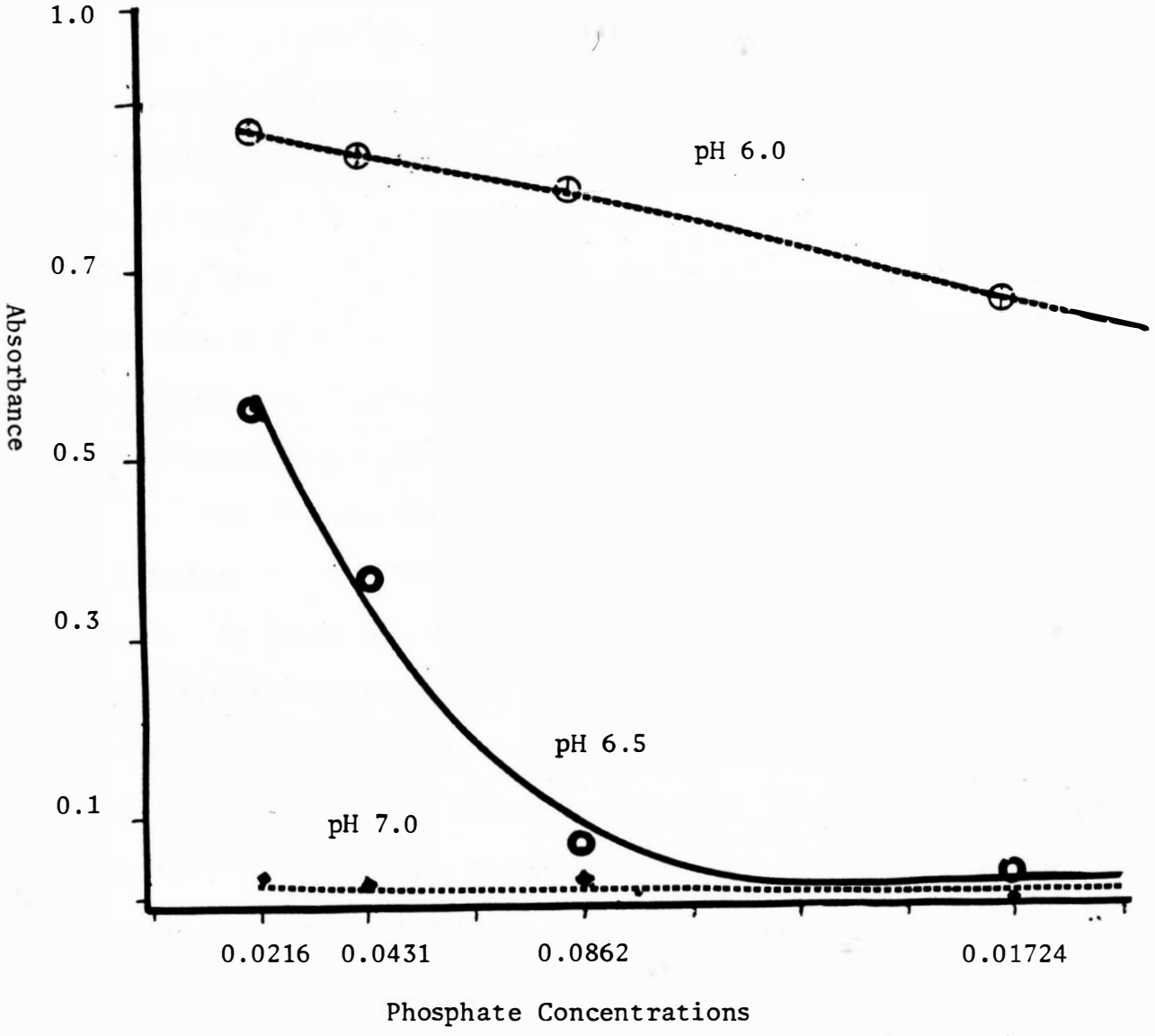


Figure 3-1. Phosphate inhibition of turbidity from polysilicic acid-protein complex formation at various pH values.

polysilicic acid complex formation was negligible at pH 7.0 (mean light absorbance < 0.015) and was highest at pH 6.0 (mean light absorbance > 0.669). Although light absorbance in solutions at pH 6.0 was decreased ($P < 0.01$) by increasing the phosphorus concentration, the difference was slight due to high precipitation in all of the reaction mixtures at this pH. These data are in agreement with those obtained by Schreier (79) who found that the amount of precipitate formed was highest at a pH of 6.0 with a mean light absorbance of 0.750.

The Figure 3-1 shows the optimum pH for phosphorus inhibition of the polysilicic acid-protein complex to be pH 6.5. At that pH, large differences existed among the four phosphorus concentrations regarding their capacity for preventing formation of the insoluble complex. At the 0.0216 N phosphate concentration, there was a high turbidity with a mean light absorbance of 0.560, but at a phosphorus concentration of 0.1724 N there was a low turbidity with a mean light absorbance of only 0.015 indicating minimal protein and polysilicic acid coprecipitation. Also, there was a consistent curvilinear effect between these two extreme phosphorus concentrations.

Polysilicic acid is a Lewis acid and interacts with a number of Lewis bases, such as some compounds containing active, lone pair electrons on oxygen or

nitrogen. Bailey (8) suggested that the interaction between protein and polymerized silicic acid is probably due to the formation of hydrogen bonding between the silanol groups of the silicic acid polymers and the secondary amide groups of the protein.

Phosphate inhibition of the protein-polysilicic acid complex is consistent with the results of the *in vivo* rat study in which supplemental dietary phosphorus reduced the incidence of silica urinary calculi. Also, if Bailey's suggestion is correct, the effect of phosphate here may involve hydrogen bonding with the secondary amide groups of the protein, thus blocking the formation of polysilicic acid-protein complex.

## DEVELOPMENT OF A SHEEP MODEL FOR THE STUDY OF SILICA URINARY CALCULI

Attempts to experimentally produce siliceous urinary calculi through the feeding of inorganic silica sources have generally been unsuccessful (20, 31). Although siliceous urinary calculi have been successfully produced in rats by feeding a diet containing 2% TES (30), attempts by Schreier and Emerick (unpublished data) to feed sheep diets containing TES showed it to be toxic. This was believed to be due to absorption of the intact TES from the rumen before it reached the gastric abomasum

where hydrolysis would occur. The experiment described here represents a method to experimentally produce siliceous urinary calculi in sheep by giving silica drinking water prepared by the acid hydrolysis of TES.

The siliceous drinking waters initially had concentrations of 1500 ppm and 3000 ppm molybdate-reactive silica, respectively, and the concentrations decreased due to silica polymerization, which in turn was believed to be due to the fairly high ionic strength of Brookings city water. The rate of decrease of the concentrations of molybdate-reactive silica can be seen in Figure 3-2. Twenty-four hours after preparation, the concentrations of molybdate-reactive silica were 1044 ppm and 1204 ppm, respectively, for the two high-silica waters.

Data from the experiment are shown in Table 3-3. The toxicological effect of the high silica levels in drinking water seemed negative. No effects of the high silica water other than calculi formation were observed, and sheep grew normally during the experimental period. The weight gains showed no significant difference due to treatment. The weight changes measured during the experimental period are shown in Appendix (Table 31).

The numbers of sheep developing urinary calculi in the control, 1500 ppm and 3000 ppm silica groups were 0, 2 (9%) and 10 (45%), respectively, and appeared to be

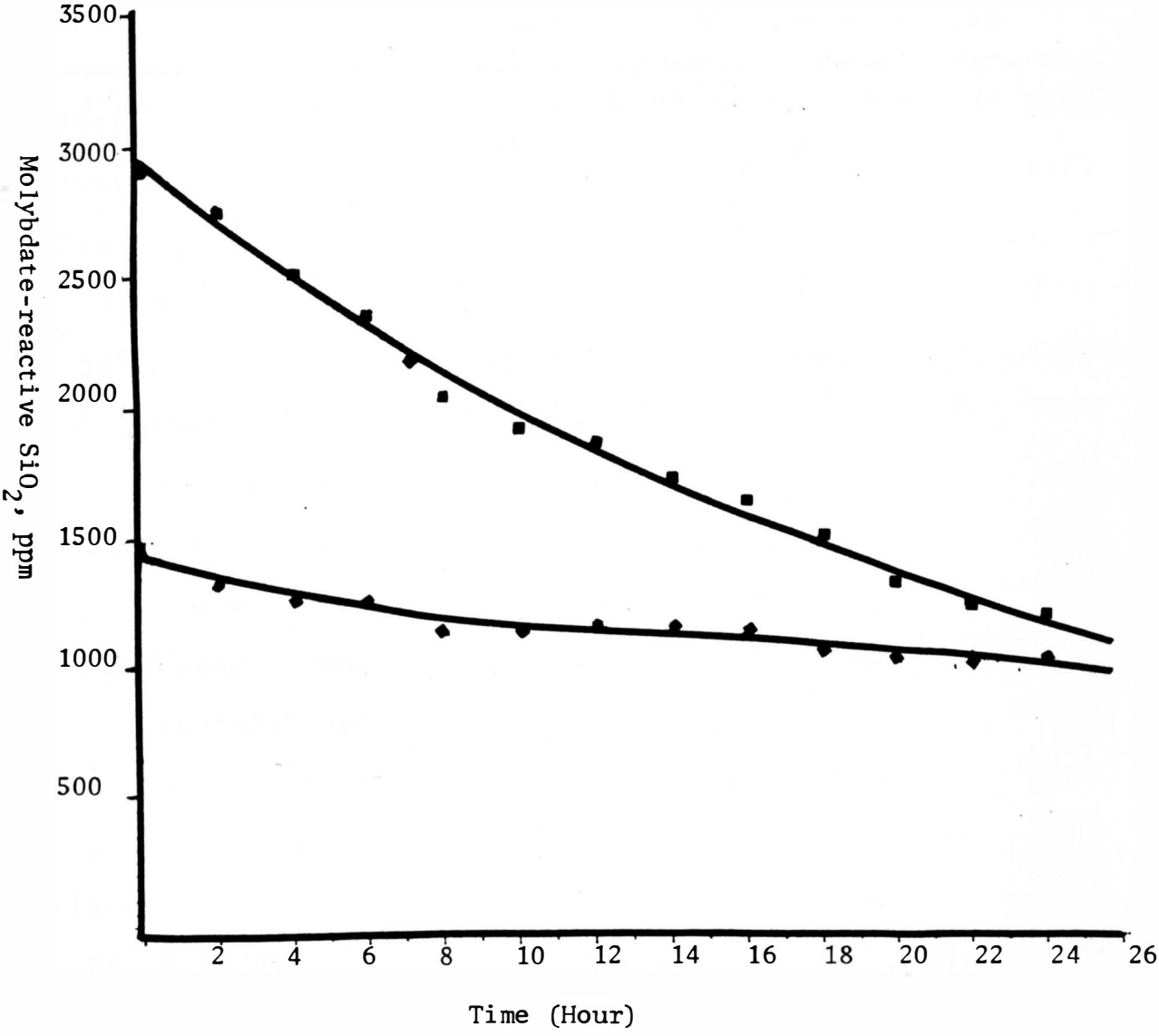


Figure 3-2. Time curves of the concentrations of molybdate-reactive $SiO_2$ in sheep drinking waters.

Table 3-3. Effect of Silica Drinking Water on Sheep[1,2,3]

| | Treatments (Silica) | | | |
|---|---|---|---|---|
| | Control | 1500 ppm | 3000 ppm | SE |
| Final weight (Kg) | 45.0 | 45.0 | 44.2 | 1.08 |
| Water consumption (L/d) | 34.1 | 33.6 | 34.4 | 0.77 |
| Urine: | | | | |
| Volume-24h (ml) | 875 | 1167 | 904 | 89.2 |
| pH[a] | 8.06 | 8.42 | 8.54 | 0.08 |
| $SiO_2$-conc. (ppm) | 283 | 267 | 319 | 26.8 |
| -total[b] (mg/d) | 197 | 266 | 243 | 16.9 |
| Ca -conc. (ppm) | 25.9 | 13.5 | 16.1 | 4.20 |
| -total[c] (mg/d) | 153 | 124 | 92 | 16.8 |
| Mg -conc. (ppm) | 65.0 | 49.3 | 58.8 | 5.82 |
| -total[c] (mg/d) | 453 | 486 | 392 | 25.2 |
| P -conc.[d] (ppm) | 2.51 | 1.34 | 1.47 | 0.34 |
| -total[e] (mg/d) | 21.0 | 14.7 | 11.3 | 4.07 |
| Blood: | | | | |
| Ca[f] (mg/dl) | 10.9 | 10.6 | 10.5 | 0.09 |
| Mg (mg/dl) | 2.20 | 2.26 | 2.25 | 0.04 |
| P (mg/dl) | 7.43 | 7.24 | 7.17 | 0.19 |
| Urinary calculi incidence[g] | 0 | 2 | 10 | |
| $SiO_2$ % in calculi ash (Ash = 72.1% of the calculi) | -- | 95.9% | 86.9% | |

(Notes for Table 3-3, continued)

[1]The treatments were three levels (0 ppm, 1500 ppm and 3000 ppm) of molybdate-reactive silica drinking water prepared by the hydrolysis of tetraethylorthosilicate (TES) at pH 3.0.

[2]Twenty two sheep per treatment, average wt=39.1 $\pm$ 4.5Kg.

[3]Statistical effects are by analysis of variance: [a]Silica effect, 0 ppm differs from 1500 ppm and 3000 ppm ($P<0.01$). [b]Silica effect, 0 ppm differs from 1500 ppm and 3000 ppm ($P<0.01$ and $P=0.056$, respectively). [c]Silica effect, 0 ppm differs from 3000 ppm ($P=0.01$). [d]Silica effect, 0 ppm differs from 1500 ppm and 3000 ppm ($P<0.05$). [e]Silica effect, 0 ppm differs from 3000 ppm ($P=0.1$). [f]Silica effect, 0 ppm differs from 1500 ppm and 3000 ppm ($P<0.01$). [g]Silica effect, using Chi-square orthogonal comparisons, 0 + 1500 ppm differs from 3000 ppm ($P<0.01$).

* * *

related to the levels of silica in the drinking water. Using chi-square orthogonal comparison, 0 and 1500 ppm $SiO_2$ groups differed ($P < 0.01$) from the 3000 ppm $SiO_2$ group. The silica calculi were found in the kidneys and none were found in the urinary bladders. The calculi were small in size ranging from 0.1 to 26 mg per sheep. The percent ash of the calculi was 72, and about 91% of the ash was silica.

There was no significant difference in water consumption due to the treatments. The water consumption was measured as liters per pen per day and the data are shown in Appendix (Table 32).

Urine volumes collected at the third, fifth and eighth weeks are shown in Appendix (Table 33). There were some variations in the urine volumes due to a few extremely high individual values but the differences were not statistically significant.

Urinary pH was increased ($P < 0.01$) from a mean of 8.06 for the control group to means of 8.43 for the 1500 ppm $SiO_2$ group and 8.54 for the 3000 ppm $SiO_2$ group (Table 3-3). With use of the rat model system for silica urinary calculi studies, there has been a similar increase in urine pH due to the feeding of TES in one instance (80) but not in another (33). Alkalinity of urine is known to be caused by an excess of $Na^+$, $K^+$, $Ca^{++}$, $Mg^{++}$ or $NH_4^+$

cations over $HPO_4^{=}$, $SO_4^{=}$, $Cl^{-}$ or $HCO_3^{-}$ anions. Related to these possibilities, only urinary calcium, magnesium and phosphorus were measured in this experiment. While both urinary phosphorus concentration and total phosphorus excretion were lower in response to high $SiO_2$ levels in the water, total urinary calcium and magnesium excretions were also lower. These data fail to provide a basis for explaining the difference in urine pH observed among the treatments in this study.

The total urinary $SiO_2$ excreted in 24 hours was increased ($P < 0.01$, 1500 ppm $SiO_2$; $P < 0.05$, 3000 ppm $SiO_2$) by the siliceous drinking waters (Table 3-3). However, the urinary $SiO_2$ concentration was not significantly increased.

Other researchers working with a number of species have shown urinary excretion of silicic acid to be increased with increasing intakes of siliceous substances, and it appeared to reach a maximum that was not exceeded by increasing the dose (47, 52, 54, 55, 58, 68, 78). Sheep diets used in this experiment contained about 1.72% silica, mostly from the grass hay. This may have limited the net absorption of additional silica. Increases in urolith formation attributed to consumption of siliceous drinking water, without accompanying increases in urinary silica concentrations, lends support to the previous

conclusion of Emerick et al (32) that siliceous urinary calculi formation is "an effect not entirely dependent upon a high level of urinary silica excretion".

Compared to the controls, total 24-h excretions of urinary calcium and magnesium were lowered ($P < 0.01$) by the siliceous drinking water. Urinary phosphorus concentrations were also lower ($P < 0.05$) and 24-hour urinary phosphorus excretion tended to be lower for the 3000 ppm $SiO_2$ group ($P = 0.1$). These results are in agreement with a decrease in urinary calcium, magnesium, and phosphorus reported for rats fed TES (33, 80).

Blood calcium was lowered ($P < 0.01$) by 1500 ppm and 3000 ppm $SiO_2$. There was no significant effect of treatments on blood magnesium or phosphorus.

Phosphate was shown *in vitro*, as described earlier, to inhibit formation of insoluble protein-polysilicic acid complexes believed to be important to silica urolith formation. Further, dietary phosphate and urine acidifying salts reduced the incidence of silica uroliths in rat studies described in this thesis and elsewhere (33, 80). Therefore, it is concluded that factors other than increases in urinary silica concentrations, most probably increases in urinary pH and decreases in urinary phosphorus, were instrumental in increasing silica urolith formation in sheep given

siliceous drinking water.

This method of producing silica urinary calculi in sheep appears to provide a model for extending results of previous rat studies to ruminants.

## SUMMARY

### 1. SYNERGISM OF DIETARY PHOSPHATE AND URINE ACIDIFYING SALTS IN PREVENTING SILICA UROLITHIASIS IN RATS

The model system described by Emerick et al. (32) using Sprague-Dawley male rats fed diets containing 2% of tetraethylorthosilicate (TES) was used. Six dietary treatments were used in a 2 x 3 factorial arrangement with two concentrations of ammonium chloride, 0 and 0.067 equivalents/kg of diet and three concentrations of dietary phosphorus, 0, 0.15% and 0.30% from $Na_2HPO_4$. The analysis of variance utilized a model consisting of ammonium chloride and phosphorus effects, and ammonium chloride x phosphorus interaction.

The concentration of 0.067 equivalents/kg diet of ammonium chloride, factored with three levels of dietary phosphorus supplementation in this experiment, was selected as being below the minimum effective concentration for reduction of silica urinary calculi.

Uroliths from all groups were found to contain 79% ash and have silica contents approximately 100 % of the ash.

Mean urine pH values in all ammonium chloride treatments were 7.14-7.16, and treatments without ammonium chloride had mean pH values of 7.44-7.53. The urine pH

was decreased ($p < 0.01$) by addition of ammonium chloride. However, supplemental phosphorus had no significant effect on urine pH. Thus, when compared with the 50% urolith incidence in the controls, reductions to 25% incidence by 0.15% added phosphorus ($P = 0.08$) and 10% incidence by 0.30% added phosphorus ($p < 0.05$) can not be attributed to differences in urine pH. However, an absence of uroliths in rats fed 0.15% or 0.30% supplemental phosphorus in combination with ammonium chloride indicates a greater antiurolithic effectiveness for supplemental phosphorus when fed under conditions that also reduce urine pH.

Based on the data from this experiment, it is concluded that supplemental dietary phosphate and urinary acidifying salts independently reduce silica urolith formation and their effects appear to be synergistic.

## 2. IN VITRO STUDIES ON THE EFFECT OF PHOSPHORUS ON PRECIPITATION OF A POLYSILICIC ACID-PROTEIN COMPLEX

In the experiment, the protein source was bovine serum albumin. The stock solutions of pH 6.0, 6.5 and 7.0 phosphate buffers were produced so that when they were diluted to 1:20 with a hydrolyzed TES solution, a constant ionic strength of 0.1724 N was obtained.

By acid hydrolysis of TES at pH 3, a 600 ppm $SiO_2$ solution was prepared for use as a source of silica in

22 lambs per treatment and 11 lambs per pen. The treatments consisted of acidic drinking water containing 3 levels of silicic acid (0 ppm, 1500 ppm and 3000 ppm). The water was heavily stirred for 2 hours in hydrolysis of the TES. The silica drinking waters initially had concentrations of 1500 and 3000 ppm molybdate-reactive silica, respectively, but the concentrations decreased due to the silica polymerization.

The toxicological effect of the high silica levels in drinking water seemed negative. No effects of the high-silica water, other than calculi formation, were observed and sheep grew normally during the experimental period. Weight gains showed no significant difference due to treatments.

The numbers of sheep developing urinary calculi in the control group, 1500 ppm silica group and 3000 ppm silica group were 0, 2 (9%) and 10 (45%), respectively, and appeared to be related to the levels of silica in drinking water. These results indicate that this is a procedure that can be used as a sheep model system.

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# APPENDIX

## Table 1

Analysis of variance table for 4 week weight; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 118 | 43924.436 | | | |
| $NH_4Cl$ | 1 | 3.374 | | 0.01 | 0.925 |
| P | 2 | 754.498 | | 1.00 | 0.372 |
| $NH_4Cl$ x P | 2 | 419.275 | | 0.55 | 0.576 |
| Error | 113 | 42738.157 | 378.213 | | |

## Table 2

Analysis of variance table for 8 week weight; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 117 | 182902.957 | | | |
| $NH_4Cl$ | 1 | 336.438 | | 0.22 | 0.644 |
| P | 2 | 5122.601 | | 1.64 | 0.199 |
| $NH_4Cl$ x P | 2 | 2365.230 | | 0.76 | 0.471 |
| Error | 112 | 174961.713 | 1562.158 | | |

Table 3

Analysis of variance table for water intake ;
(rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 353 | 33537.749 | | | |
| $NH_4Cl$ | 1 | 60.143 | | 0.65 | 0.421 |
| P | 2 | 716.407 | | 3.86 | 0.022 |
| $NH_4Cl$ x P | 2 | 478.851 | | 2.58 | 0.077 |
| Error | 348 | 32267.493 | 92.722 | | |

Table 4

Analysis of variance table for urine volume;
(rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 143 | 10492.826 | | | |
| $NH_4Cl$ | 1 | 2.006 | | 0.03 | 0.868 |
| P | 2 | 483.513 | | 3.35 | 0.039 |
| $NH_4Cl$ x P | 2 | 34.013 | | 0.24 | 0.791 |
| Error | 138 | 9973.291 | 72.270 | | |

Table 5

Analysis of variance table for urine pH;
(rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 143 | 6.857 | | | |
| $NH_4Cl$ | 1 | 3.737 | | 171.65 | 0.001 |
| P | 2 | 0.071 | | 1.64 | 0.199 |
| $NH_4Cl$ x P | 2 | 0.043 | | 1.00 | 0.371 |
| Error | 138 | 3.005 | 0.021 | | |

Table 6

Analysis of variance table for urine silica;
(rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 145 | 3395493.486 | | | |
| $NH_4Cl$ | 1 | 1550.391 | | 0.07 | 0.793 |
| P | 2 | 88156.907 | | 1.97 | 0.143 |
| $NH_4Cl$ x P | 2 | 172467.749 | | 3.86 | 0.023 |
| Error | 140 | 3130022.240 | 22357.301 | | |

Table 7

Analysis of variance table for urine calcium; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 146 | 6000.192 | | | |
| $NH_4Cl$ | 1 | 26.059 | | 0.95 | 0.331 |
| P | 2 | 1950.087 | | 35.65 | 0.001 |
| $NH_4Cl$ x P | 2 | 178.389 | | 3.26 | 0.041 |
| Error | 141 | 3855.894 | 27.346 | | |

Table 8

Analysis of variance table for urine magnesium; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 146 | 11496.578 | | | |
| $NH_4Cl$ | 1 | 10.884 | | 0.16 | 0.688 |
| P | 2 | 1944.871 | | 14.42 | 0.001 |
| $NH_4Cl$ x P | 2 | 28.094 | | 0.20 | 0.818 |
| Error | 141 | 9506.394 | 67.421 | | |

Table 9

Analysis of variance table for urine phosphorus; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 143 | 514523.937 | | | |
| $NH_4Cl$ | 1 | 0.562 | | 0.00 | 0.982 |
| P | 2 | 364581.375 | | 168.19 | 0.001 |
| $NH_4Cl$ x P | 2 | 371.625 | | 0.17 | 0.843 |
| Error | 138 | 149570.375 | 1083.843 | | |

Table 10

Analysis of variance table for blood plasma calcium; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 58 | 6.201 | | | |
| $NH_4Cl$ | 1 | 1.012 | | 11.65 | 0.001 |
| P | 2 | 0.294 | | 1.70 | 0.193 |
| $NH_4Cl$ x P | 2 | 0.276 | | 1.59 | 0.213 |
| Error | 53 | 4.606 | 0.086 | | |

Table 11

Analysis of variance table for blood plasma magnesium; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 58 | 1.645 | | | |
| $NH_4Cl$ | 1 | 0.080 | | 2.87 | 0.096 |
| P | 2 | 0.061 | | 1.09 | 0.344 |
| $NH_4Cl$ x P | 2 | 0.010 | | 0.18 | 0.838 |
| Error | 53 | 1.493 | 0.028 | | |

Table 12

Analysis of variance table for blood plasma phosphorus; (rat experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 58 | 27.769 | | | |
| $NH_4Cl$ | 1 | 1.404 | | 3.10 | 0.084 |
| P | 2 | 2.244 | | 2.48 | 0.093 |
| $NH_4Cl$ x P | 2 | 0.192 | | 0.21 | 0.809 |
| Error | 53 | 23.941 | 0.451 | | |

Table 13

Analysis of variance table for pH 6.0 study;
(silicic acid-protein experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 31 | 0.215 | | | |
| Phosphate | 3 | 0.188 | | 64.94 | 0.001 |
| Error | 28 | 0.027 | 0.001 | | |

Table 14

Analysis of variance table for pH 6.5 study;
(silicic acid-protein experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 31 | 1.710 | | | |
| Phosphate | 3 | 1.643 | | 227.91 | 0.001 |
| Error | 28 | 0.067 | 0.002 | | |

Table 15

Analysis of variance table for pH 7.0 study;
(silicic acid-protein experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 23 | 0.0002 | | | |
| Phosphate | 3 | 0.0001 | | 5.86 | 0.005 |
| Error | 20 | 0.0001 | 0.000006 | | |

Table 16

Analysis of variance table for final weight;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 64 | 1534.216 | | | |
| Treatment | 2 | 9.949 | | 0.19 | 0.825 |
| Error | 59 | 1524.267 | 25.835 | | |

Table 17

Analysis of variance table for water intake;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 29 | 163.846 | | | |
| Treatment | 2 | 3.764 | | 0.32 | 0.731 |
| Error | 27 | 160.082 | 5.928 | | |

Table 18

Analysis of variance table for urine volume;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 24296727.827 | | | |
| Treatment | 2 | 1379615.415 | | 2.71 | 0.072 |
| Error | 90 | 22917112.412 | 254634.582 | | |

Table 19

Analysis of variance table for urine pH;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 24.960 | | | |
| Treatment | 2 | 3.932 | | 8.41 | 0.001 |
| Error | 90 | 21.028 | 0.233 | | |

Table 20

Analysis of variance table for urine $SiO_2$ conc.
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 2111598.924 | | | |
| Treatment | 2 | 45856.538 | | 1.00 | 0.372 |
| Error | 90 | 2065742.386 | 22952.693 | | |

Table 21

Analysis of variance table for total urine $SiO_2$; (sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 900847.311 | | | |
| Treatment | 2 | 76066.040 | | 4.15 | 0.019 |
| Error | 90 | 824781.270 | 9164.236 | | |

Table 22

Analysis of variance table for urine Ca conc; (sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 53429.647 | | | |
| Treatment | 2 | 2513.280 | | 2.22 | 0.114 |
| Error | 90 | 50916.367 | 565.737 | | |

Table 23

Analysis of variance table for total urine Ca;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 875669.118 | | | |
| Treatment | 2 | 57424.564 | | 3.16 | 0.047 |
| Error | 90 | 818244.553 | 9091.606 | | |

Table 24

Analysis of variance table for urine Mg conc;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 100878.903 | | | |
| Treatment | 2 | 3277.210 | | 1.51 | 0.226 |
| Error | 90 | 97601.692 | 1084.463 | | |

Table 25

Analysis of variance table for total urine Mg;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 1983439.311 | | | |
| Treatment | 2 | 151119.973 | | 3.71 | 0.028 |
| Error | 90 | 1832319.338 | 20359.103 | | |

Table 26

Analysis of variance table for urine P conc;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 364.296 | | | |
| Treatment | 2 | 24.378 | | 3.23 | 0.044 |
| Error | 90 | 339.918 | 3.776 | | |

Table 27

Analysis of variance table for total urine P;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 92 | 49066.016 | | | |
| Treatment | 2 | 1423.937 | | 1.34 | 0.266 |
| Error | 90 | 47642.079 | 529.356 | | |

Table 28

Analysis of variance table for blood Ca;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 63 | 12.487 | | | |
| Treatment | 2 | 2.257 | | 6.73 | 0.002 |
| Error | 61 | 10.229 | 0.167 | | |

Table 29

Analysis of variance table for blood Mg;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 63 | 1.707 | | | |
| Treatment | 2 | 0.033 | | 0.61 | 0.548 |
| Error | 61 | 1.674 | 0.027 | | |

Table 30

Analysis of variance table for blood P;
(sheep experiment)

| Variation | DF | Sum of squares | Mean square | F value | Prob |
|---|---|---|---|---|---|
| Total | 63 | 48.203 | | | |
| Treatment | 2 | 0.692 | | 0.44 | 0.643 |
| Error | 61 | 47.510 | 0.778 | | |

Table 31

Weight Gains during the Experimental Period

| Weight (Kg) | Treatments: Control Group | 1500 ppm $SiO_2$ group | 3000 ppm $SiO_2$ group | F | PR |
|---|---|---|---|---|---|
| Initial | 85.2 | 97.5 | 85.7 | 0.32 | 0.73 |
| One month | 99.4 | 102.1 | 96.8 | 0.34 | 0.71 |
| Two month | 99.2 | 99.1 | 97.3 | 0.19 | 0.83 |

Table 32

Water Consumption during the Experimental Period

| | Treatments (Silica) Control | 1500 ppm | 3000 ppm |
|---|---|---|---|
| 1st week | 35.6 | 35.6 | 38.6 |
| 2nd week | 35.2 | 33.9 | 34.5 |
| 3rd week | 34.5 | 33.2 | 33.3 |
| 4th week | 30.4 | 30.7 | 31.5 |
| 6th week | 34.8 | 34.7 | 34.3 |

Table 33

Urine Volumes (L) in Three Collections

| | Treatments (Silica) | | | | |
|---|---|---|---|---|---|
| | Control | 1500 ppm | 3000 ppm | F | PR |
| 3rd week | 850 | 1173 | 731 | 2.77 | 0.09 |
| 5th week | 1108 | 1400 | 1427 | 0.81 | 0.46 |
| 8th week | 765 | 1020 | 738 | 1.71 | 0.19 |